Supplementary information

Characterization of CAR specific for antigens CD19 and CD20.

We have constructed novel chimeric receptors specific for antigens CD19 and CD20 using mouse hybridomas MEM87 and B2-D3 which produce mAb against CD20 and CD19, respectively. For details of the procedure see M&M. The scheme of our CAR constructs is depicted in **Fig. S1**, the numbers indicate amino-acid residues of respective human protein chain. The data in **Fig. S2** shows the expression of the constructs at the protein level, the plasmid DNA was transfected into HEK cells and the expression of the protein was detected in lysates by immunoblotting using the anti-TCRzeta mAb, the size of the CAR protein is around 60kD (marker not shown) as expected. Next, we analysed the surface expression of the CAR construct using flow-cytomery in human T-cells. First, The CAR sequence was cloned into the lentiviral vector pWPXLd and viral particles were then produced and used to infect human T-cells as described in M&M, cells were then expanded in vitro. The images in **Fig. S3** shows the surface expression of CAR19 and CAR20 which was revealed with goat anti-mouse serum and the expression is compared to uninfected cells. To demonstrate the functionality of the construct we performed flow-cytometry based in vitro cytotoic test (**Fig. S4**), the data shows that the CFSE-labeled targeT-cells Ramos were efficiently killed by CAR19 and CAR20 T-cells while no significant lysis was observed in control uninfected T-cells.

Fig. S1

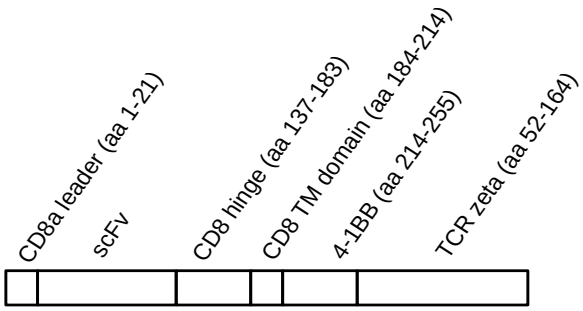


Fig. S2

19 20 Neg.

Fig. S3

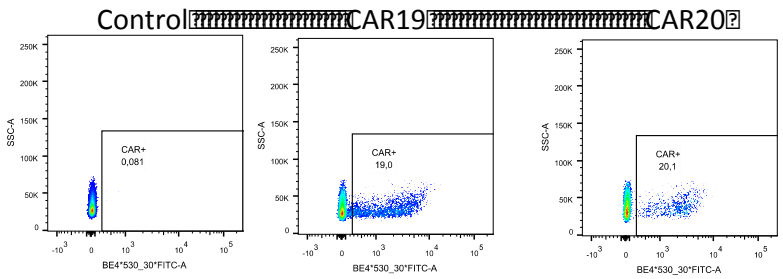
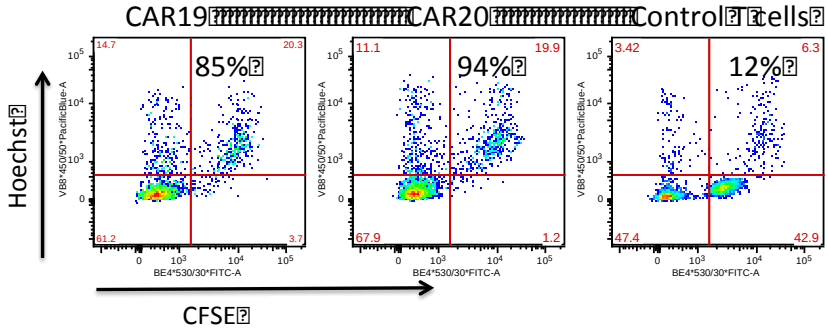

Control
CAR19
CAR20
SSC-A
CAR+
0,081
CAR+
19,0
CAR+
20,1
BE4*530_30*FITC-A

Fig. S4


CAR19
CAR20
Control T cells
Hoechst
VB8*450/50*PacificBlue-A
BE4*530/30*FITC-A
CFSE
85%
94%
12%
14.7
20.3
61.2
3.7
11.1
19.9
67.9
1.2
3.42
6.3
47.4
42.9